Claims 43 and 45 have been canceled. Claims 42 and 44 have been amended to include the recitations of Claims 43 and 45.

New Claims

New Claims 50-90 have been added. Claims 50-90 comprise previously filed Claims 1-49 further including the recitations of Claim 42. No new matter has been added by the new claims.

Rejections of Claims under 35 U.S.C. §112, Second Paragraph

Claims 1-25, 27-36, 38, 40, and 42-49 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention, for the reasons stated below and in the Office action of paper no. 4.

A. The Examiner states that "Claims 1-25, 27 and 42-49 are indefinite over the recitation of the terms 'identifying' and 'identify' in claims 1, 14, 42, 44, and 46-48" (Office Action, page 2). The Examiner further states that "the portion of the specification cited by applicant does not clarify whether the recitation of 'identifying'/'identify' would in fact require any type of active step, or whether steps of 'identifying' might encompass, e.g., thought processes" (Office Action, page 3).

Respectfully, Applicants disagree. However, solely to advance prosecution, Claims 1, 14, 42, 44 and 46-48 have been amended to recite the active steps of "detecting" and/or "detect".

B. The Examiner states that "Claims 1-25, 27-36, 38, 40, 42-45 and 48-49 are indefinite over the recitation of the terms 'analyzing' and 'analyzed' in claims 1, 14, 28, 42, 44, and 48" (Office Action, page 3). The Examiner states that "the portion of the specification cited by applicant does not clarify whether the recitation of 'analyzing' and 'analyzed' would in fact require any type of active step, or whether steps of 'analyzing' might encompass, e.g., thought processes" (Office Action, page 4).

Respectfully, Applicants disagree. However, solely to advance prosecution, Claims 1, 14, 28, 42, 44 and 48 have been amended to delete "analyzing" and "analyzed" and include active steps.

C. The Examiner states that "Claims are indefinite over the recitation of the terms 'selected'and 'selecting' in claims1, 12, 14, 24, 28, 31, and 46-48" (Office Action, page 4). The Examiner states that "the portion of the specification cited by applicant does not clarify whether the recitation of 'selected' and 'selecting' would in fact require any type of active step, or whether steps of 'selecting' might encompass, e.g., thought processes" (Office Action, page 5).

Respectfully, Applicants disagree. However, solely to advance prosecution, Claims 1, 12, 14, 24, 28, 31, and 46-48 have been amended to recite the active steps of "isolating" and/or "isolated".

D. The Examiner states that "Claims 42-45 are indefinite over the recitation of the limitation'proposed pairs' in claims 42 and 44". The Examiner states that "it is unclear as to what types of pairs might be considered to be 'proposed' pairs, and as to how such pairs relate back to the 'pairs of fragments' of claims 1 and 14" (Office Action, page 5).

Respectfully, Applicants disagree. However, solely to advance prosecution, Claims 42 and 44 have been amended to delete "proposed".

E. The Examiner states that "Claims 42-45 are indefinite over the recitation of the term 'candidate single nucleotide polymorphisms' in claims 42 and 44". The Examiner states that "[i]t remains unclear as to how a 'candidate single nucleotide polymorphism' would differ from a 'single nucleotide polymorphism' within the context of the claimed invention" (Office Action, page 6).

Respectfully, Applicants disagree. However, solely to advance prosecution, Claims 42 and 44 have been amended to delete "candidate".

F. The Examiner states that "Claims 42-45 are indefinite over the recitation of the phrase 'determining the number of candidate matches for the same chromosomal location, wherein said

candidate matches are accepted if said number of matches does not exceed expectations'" (Office Action, page 6). The Examiner states that "[w]hile the portion of the specification referenced by the applicant provides an example of how putative orthologues may be analyzed to determine whether a 'candidate match' would constitute a 'match', no particular limitations or definitions are provided that would provide a clear limitation of the recitation in the present claims of the language 'if said number of matches does not exceed expectations' " (Office Action, page 7).

Applicants have amended Claims 42 and 44 to include the recitations of Claims 43 and 45. Specifically, Claims 42(e) and 44(e) recite "determining the number of single nucleotide polymorphism matches for the same chromosomal location, wherein said matches are accepted if said number of matches does not exceed the theoretical expectations of a binomial or Poisson distribution, wherein accepted matches are considered a pair".

G. The Examiner states that "Claims 43 and 45 are indefinite over the recitation of the phrase 'wherein said expectations are determined according to binomial or Poisson distribution'" (Office Action, page 7). The Examiner states that "the present rejection was made because applicants' claim language does not make clear how one is to employ 'binomial or Poisson distributions' to determine expectations" (Office Action, page 7).

Claims 43 and 45 have been canceled. Claims 42 and 44 have been amended to recite "wherein said matches are accepted if said number of matches does not exceed the theoretical expectations of a binomial or Poisson distribution." Thus, Applicants have clearly stated how one of skill is to employ binomial and Poisson distributions. Furthermore, Applicants disclose how to calculate and use the statistical expectations to select a pair (Examples 1-3), and the skilled artisan is well-versed in the use of such statistical tools.

All claims, particularly as amended, are in condition for allowance. Respectfully, Applicants request the rejection be withdrawn.

Rejection of Claims 1-4, 8-10, 12-17, 21-22, 24-25, 27-29, 31-32 and 46-49 under 35 U.S.C. §102(b)

Claims 1-4, 8-10, 12-17, 21-22, 24-25, 27-29, 31-32 and 46-49 are rejected under 35 U.S.C. 102(b) as being clearly anticipated by Gu *et al.* (*BioTechniques* 24(5):836-837 [5/1998]), for the reasons stated in the Office Action of paper no. 4. The Examiner states that "[t]he step of obtaining amplification products taught by Gu et al. constitutes a step of obtaining a 'nucleic acid-containing sample' that is encompassed by the present claims" (Office Action, page 9).

Applicants note that Claims 1, 14, 28, 46, 47 and 48 have been amended to recite "obtaining a **non-PCR amplified** nucleic acid-containing sample" (emphasis added). As described in the Amendment filed March 13, 2001, Gu *et al.* do not teach obtaining a non-PCR amplified nucleic acid-containing sample. Therefore, Gu *et al.* do not teach every element of the claimed invention, particularly as amended, as required under 35 U.S.C. 102. Reconsideration and withdrawal of the rejection are respectfully requested.

Rejection of Claims 6-7, 19-20, 36, 38, and 40 under 35 U.S.C. §103(a)

Claims 6-7, 19-20, 36, 38, and 40 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gu *et al.* (*BioTechniques* 24(5):836-837 [5/1998]) in view of Landegren *et al.* (*Genome Res.* 8(8):769-776 [8/1998]), for reasons stated in the Office Action of paper no. 4 (Office Action, page 9). The Examiner states that "the claims encompass 'obtaining' in any manner any type of 'nucleic acid-containing sample'" and that "[a]ccordingly, methods in which 'obtaining' is achieved by steps including amplification are encompassed by the present claims" (Office Action, pages 10-11).

Applicants note that Claims 1, 14, 28, 46, 47 and 48 have been amended to recite "obtaining a **non-PCR amplified** nucleic acid-containing sample" (emphasis added). As described above, Gu *et al.* does not anticipate or render obvious Claims 1-4, 8-10, 12-17, 21-22, 24-25, 27-29, 31-32 and 46-49, particularly as amended. Since Claims 6-7, 19-20, 36, 38 and 40 incorporate all of the limitations of the claims from which they depend, Gu *et al.* also does not anticipate or render obvious Claims 6-7, 19-20, 36, 38 and 40.

Moreover, the addition of Landegren *et al.* does not remedy the defects of Gu *et al.* As stated in the Amendment filed March 13, 2001, Landegren *et al.* teach that all SNP-related

methods require PCR amplification of known target sequences. Thus, the teachings of Landegren *et al.* further support the disclosure of Gu *et al.*, i.e., that PCR amplification is a necessary component of SNP discovery methods prior to the subject invention. Therefore, neither Landegren *et al.* nor Gu *et al.*, either alone or in combination, teach or suggest obtaining a **non-PCR amplified** nucleic acid-containing sample. Thus, Landegren *et al.* and Gu *et al.* do not provide the teaching or suggestion to make the claimed combination, do not provide the reasonable expectation of success if one of ordinary skill were to do so, and do not teach or suggest all claim limitations of Applicants' invention, as required under 35 U.S.C. §103. Reconsideration and withdrawal of the rejection are respectfully requested.

Rejection of Claims 5 and 18 under 35 U.S.C. §103(a)

Claims 5 and 18 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gu *et al.* in view of Wu *et al.* (*DNA* 8(2):135-142 [1989]), for the reasons stated in the Office Action of paper no. 4. The Examiner states that "the present claims do not exclude the use of nucleic acids of known sequence, and the claims encompass 'obtaining' in any manner any type of 'nucleic acid-containing sample'" and that "[a]ccordingly, methods in which 'obtaining' is achieved by steps including amplification using 'specific PCR primers' are encompassed by the present claims" (Office Action, page 12).

Applicants note that Claims 1, 14, 28, 46, 47 and 48 have been amended to recite "obtaining a **non-PCR amplified** nucleic acid-containing sample" (emphasis added). As described above, Gu *et al.* does not anticipate or render obvious Claims 1-4, 8-10, 12-17, 21-22, 24-25, 27-29, 31-32 and 46-49, particularly as amended. Since Claims 5 and 18 incorporate all of the limitations of the claims from which they depend, Gu *et al.* also does not anticipate or render obvious Claims 5 and 18.

The addition of Wu *et al.* does not remedy the defects of Gu *et al.* As stated in the Amendment filed March 13, 2001, Wu *et al.* does not teach a method for identifying new polymorphisms by analysis of RNA, but rather teach the use of a known polymorphism-specific probe on a spot of total RNA isolated from an individual to determine its presence or absence. Therefore, neither Gu *et al.* nor Wu *et al.*, either alone or in combination, teach or suggest a method for detecting a collection of polymorphisms by analysis of RNA comprising obtaining a

non-PCR amplified nucleic acid-containing sample. Thus, Gu *et al.* and Wu *et al.* do not provide the teaching or suggestion to make the claimed combination, and do not provide a reasonable expectation of success if one of ordinary skill were to do so, as required under 35 U.S.C. §103. Furthermore, even if one of ordinary skill were to use RNA in the methods of Gu *et al.*, the combination would still not teach or suggest all claim limitations of Applicants' invention, as required under 35 U.S.C. §103. Reconsideration and withdrawal of the rejection are respectfully requested.

Rejection of Claims 11, 23 and 30 under 35 U.S.C. §103(a)

Claims 11, 23, and 30 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gu *et al.* in view of Bonn *et al.* (U.S. Patent No. 5,585,236), for the reasons stated in the Office Action of paper no. 4 (Office Action, page 12). The Examiner states that "the present claims encompass 'obtaining' in any manner any type of 'nucleic acid-containing sample'" and that "[a]ccordingly, methods in which 'obtaining' is achieved by steps including amplification using 'specific PCR primers' are encompassed by the present claims" (Office Action, page 13).

Applicants note that Claims 1, 14, 28, 46, 47 and 48 have been amended to recite "obtaining a **non-PCR amplified** nucleic acid-containing sample" (emphasis added). Again, as described above, Gu *et al.* does not anticipate or render obvious Claims 1-4, 8-10, 12-17, 21-22, 24-25, 27-29, 31-32 and 46-49, particularly as amended. Since Claims 11, 23 and 30 incorporate all of the limitations of the claims from which they depend, Gu *et al.* also does not anticipate or render obvious Claims 11, 23 and 30.

The addition of Bonn *et al.* does not remedy the defects of Gu *et al.* While Applicants do not concede that one of ordinary skill in the art would have been motivated to combine the teaching of Gu *et al.* and Bonn *et al.*, the combination would not provide a reasonable expectation of success in producing the claimed invention, as required under 35 U.S.C. §103. At most, the combination would result in isolating nucleic acid fragments using HPLC in the methods of Gu *et al.* Therefore, neither Gu *et al.* nor Bonn *et al.*, either alone or in combination, teach or suggest a method for detecting a collection of polymorphisms comprising obtaining a **non-PCR amplified** nucleic acid-containing sample and isolating a subset of nucleic acid fragments using HPLC. Thus, Gu *et al.* and Bonn *et al.* do not teach or suggest all claim

limitations of Applicants' invention, as required under 35 U.S.C. §103. Reconsideration and withdrawal of the rejection are respectfully requested.

Rejection of Claims 33-35 under 35 U.S.C. §103(a)

Claims 33-35 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gu *et al.* in view of Drmanac (U.S. Patent No. 6,025,136), for the reasons stated in the Office Action of paper no. 4. The Examiner states that "the present claims encompass 'obtaining' in any manner any type of nucleic-acid containing sample'" and that "[a]ccordingly, methods in which 'obtaining' is achieved by steps including amplification using 'specific PCR primers' are encompassed by the present claims" (Office Action, page 14).

Once again, Applicants note that Claims 1, 14, 28, 46, 47 and 48 have been amended to recite "obtaining a **non-PCR amplified** nucleic acid-containing sample". Gu *et al.* does not anticipate or render obvious Claims 1-4, 8-10, 12-17, 21-22, 24-25, 27-29, 31-32 and 46-49, particularly as amended. Since Claims 33-35 incorporate all of the limitations of the claims from which they depend, Gu *et al.* also does not anticipate or render obvious Claims 33-35.

The addition of Drmanac does not remedy the defects of Gu *et al.* Specifically, neither Gu *et al.* nor Drmanac teach or suggest a method for genotyping a nucleic acid sample comprising obtaining a non-PCR amplified nucleic acid-containing sample. While Applicants do not concede that one of ordinary skill in the art would have been motivated to combine the teaching of Gu *et al.* and Drmanac, the combination clearly does not teach or suggest all limitations of Applicants' invention, as required under 35 U.S.C. §103. Furthermore, the combination does not provide a reasonable expectation of success in producing the claimed invention. Reconsideration and withdrawal of the rejection are respectfully requested.

## CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (978) 341-0036.

Respectfully submitted,
HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By _____
Brian P. Turano
Registration No. P-50,734
Telephone (978) 341-0036
Facsimile (978) 341-0136

Concord, MA 01742-9133
Dated: 1/30/02

MARKED UP VERSION OF AMENDMENTS

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

1. (Twice Amended) A method for [identifying] detecting a collection of polymorphisms from nucleic acid molecules in a sample, [by analyzing a subset of the molecules,] consisting essentially of the steps of:

a. obtaining a non-PCR amplified nucleic acid-containing sample;

b. treating the nucleic acid molecules in said sample to produce a reduced representation of nucleic acid fragments [selected] isolated in a sequence-dependent manner by a method comprising:

i. fractionating said nucleic acid molecules to produce nucleic acid fragments; and

ii. [selecting] isolating a subset of said nucleic acid fragments,

wherein either (i) or (ii) or both (i) and (ii) are performed in a sequence-dependent manner;

c. [analyzing the reduced representation to identify] detecting pairs of fragments from the same chromosomal location in the reduced representation, wherein pairs of fragments from the same chromosomal location are orthologous sequences; and

d. comparing [pairs of] orthologous sequences to [identify] detect polymorphisms between said sequences,

thereby [identifying] detecting a collection of polymorphisms.

12. (Amended) The method of Claim 1, wherein step (b)(ii) is performed by [selecting] isolating nucleic acid fragments which hybridize to [selected] isolated additional nucleic acid sequences.

14. (Twice Amended) A method for [identifying] detecting a collection of polymorphisms from nucleic acid molecules in a sample, [by analyzing a subset of the molecules,] consisting essentially of the steps of:

a. obtaining a non-PCR amplified nucleic acid-containing sample to be assessed;

b. treating nucleic acid molecules in said sample to produce a reduced representation of nucleic acid fragments [selected] isolated in a sequence-dependent manner by a method comprising:

i. fractionating said nucleic acid molecules with one or more restriction endonucleases to produce nucleic acid fragments; and

ii. [selecting] isolating a subset of said nucleic acid fragments using size fractionation;

wherein either (i) or (ii) or both (i) and (ii) are performed in a sequence-dependent manner;

c. [analyzing the reduced representation to identify] detecting pairs of fragments from the same chromosomal location in the reduced representation, wherein pairs of fragments from the same chromosomal location are orthologous sequences; and

d. comparing [pairs of] orthologous sequences to [identify] detect polymorphisms between said orthologous sequences,

thereby [identifying] detecting a collection of polymorphisms from said nucleic acid molecules.

24. (Amended) The method of Claim 14, wherein step (b)(ii) is performed by [selecting] isolating nucleic acid fragments which hybridize to [selected] isolated additional nucleic acid sequences.

28. (Twice Amended) A method for genotyping a nucleic acid sample to determine the nucleotide present at one or more polymorphic sites of nucleic acid fragments contained in a reduced representation, consisting essentially of the steps of:

a. obtaining a non-PCR amplified nucleic acid-containing sample;

b. treating the nucleic acid molecules in said sample to produce a reduced representation of nucleic acid fragments [selected] isolated in a sequence-dependent manner by a method comprising:

i. fractionating said nucleic acid molecules to produce nucleic acid fragments; and

ii. [selecting] isolating a subset of said nucleic acid fragments,

wherein either (i) or (ii) or both (i) and (ii) are performed in a sequence-dependent manner; and

c. [analyzing the nucleic acid fragments contained in the reduced representation to assess the genotype at one or more polymorphic sites,

thereby genotyping a nucleic acid sample to determine] determining the nucleotide present at one or more polymorphic sites of nucleic acid fragments contained in the reduced representation, thereby genotyping the nucleic acid sample.

31. (Amended) The method of Claim 28, wherein step (b)(ii) is performed by [selecting] isolating nucleic acid fragments which hybridize to [selected] isolated additional nucleic acid sequences.

42. (Twice Amended) The method of Claim 1, wherein step (c) is performed by the following steps:

a. comparing the sequences of [two] pairs of fragments from the reduced representation, wherein [the two sequences are further analyzed] if the two sequences are at least 80% identical over at least 80% of the length of the shorter of the two sequences, the two sequences are then aligned;

b. aligning the two sequences identified from (a), wherein [the two sequences are further analyzed] if the two sequences are identical over 10 or more contiguous bases within each of the first 50 bases and the last 50 bases of the sequences, the two sequences are compared to determine single nucleotide polymorphisms;

c. [identifying] determining [candidate] single nucleotide polymorphisms in the sequences of (b), wherein [the two sequences are further analyzed] if the number of [candidate] single nucleotide polymorphisms does not exceed 1% of the total number of bases in the shorter of the two sequences, [wherein] the two sequences [which meet the criteria of (a) - (c)] qualify as a [candidate] match;

d. repeating (a) - (c) for all [proposed] pairs of fragments; and

e. determining the number of [candidate] matches for the same chromosomal location, wherein said [candidate] matches are accepted if said number of matches does not exceed the theoretical expectations of a binomial or Poisson distribution,

wherein accepted [candidate] matches are considered a pair.

44. (Twice Amended) The method of Claim 14, wherein step (c) is performed by the following steps:

a. comparing the sequences of [two] pairs of fragments from the reduced representation, wherein [the two sequences are further analyzed] if the two sequences are at least 80% identical over at least 80% of the length of the shorter of the two sequences, the two sequences are then aligned;

b. aligning the two sequences identified from (a), wherein [the two sequences are further analyzed] if the two sequences are identical over 10 or more contiguous bases within each of the first 50 bases and the last 50 bases of the sequences, the two sequences are compared to determine single nucleotide polymorphisms;

c. [identifying] determining [candidate] single nucleotide polymorphisms in the sequences of (b), wherein [the two sequences are further analyzed] if the number of [candidate] single nucleotide polymorphisms does not exceed 1% of the total number of bases in the shorter of the two sequences, [wherein] the two sequences [which meet the criteria of (a) - (c)] qualify as a [candidate] match;

d. repeating (a) - (c) for all [proposed] pairs of fragments; and

e. determining the number of [candidate] matches for the same chromosomal location, wherein said [candidate] matches are accepted if said number of matches does not exceed the theoretical expectations of a binomial or Poisson distribution,

wherein accepted [candidate] matches are considered a pair.

46. (Twice Amended) A method for determining a population of polymorphisms from nucleic acid molecules in a sample, consisting essentially of the steps of:

a. obtaining a non-PCR amplified nucleic acid-containing sample to be assessed;

b. treating nucleic acid molecules in said sample to produce nucleic acid fragments [selected] isolated in a sequence-dependent manner by a method comprising:

i. fractionating said nucleic acid molecules to produce nucleic acid fragments; and

ii. [selecting] isolating a subset of said nucleic acid fragments;

wherein either (i) or (ii) or both (i) and (ii) are done in a sequence-dependent manner;

c. [selecting] isolating from said subset nucleic acid fragments which occur at the same chromosomal locus, thereby producing a pair, and

d. [identifying] detecting polymorphisms between fragments of a pair;

thereby determining a population of polymorphisms from said nucleic acid-containing sample.

47. (Twice Amended) A method for determining a population of polymorphisms from nucleic acid molecules in a sample, consisting essentially of the steps of:

a. obtaining a non-PCR amplified nucleic acid-containing sample to be assessed;

b. treating nucleic acid molecules in said sample to produce nucleic acid fragments [selected] isolated in a sequence-dependent manner by a method comprising:

i. fractionating said nucleic acid molecules with one or more restriction endonucleases to produce nucleic acid fragments; and

ii. [selecting] isolating a subset of said nucleic acid fragments using size fractionation;

wherein either (i) or (ii) or both (i) and (ii) are done in a sequence-dependent manner;

c. isolating from said subset nucleic acid fragments which occur at the same chromosomal locus, thereby producing a pair, and

d. [identifying] detecting polymorphisms between fragments of a pair;

thereby determining a population of polymorphisms from said nucleic acid-containing sample.

48. (Twice Amended) A method for genotyping a nucleic acid-containing sample from an individual to determine the nucleotide present at one or more polymorphic sites, the method consisting essentially of:

a. obtaining a first non-PCR amplified nucleic acid-containing sample to be assessed;

b. treating nucleic acid molecules in said sample to produce a reduced representation of nucleic acid fragments [selected] isolated in a sequence-dependent manner by a method comprising:

i. fractionating said nucleic acid molecules to produce nucleic acid fragments; and

ii. [selecting] isolating a subset of said nucleic acid fragments;

wherein either (i) or (ii) or both (i) and (ii) are done in a sequence-dependent manner;

c. [analyzing the reduced representation to identify] detecting pairs of fragments from the same chromosomal location in the reduced representation, wherein pairs of fragments from the same chromosomal location are orthologous sequences;

d. comparing [pairs of] orthologous sequences to [identify] detect polymorphisms between the orthologous sequences;

e. obtaining a second nucleic acid-containing sample from an individual to be assessed; and

f. [analyzing said second nucleic acid-containing sample to assess the genotype at one or more polymorphisms identified in (d),

thereby genotyping a nucleic acid-containing sample from an individual to determine] determining the nucleotide present at one or more polymorphic sites identified in (d), thereby genotyping a nucleic acid-containing sample from an individual.

49. (Twice Amended) A method according to Claim 48, wherein the second nucleic acid-containing sample is a sample which has been treated by a method comprising:

i. fractionating the nucleic acid molecules in said sample to produce nucleic acid fragments; and

ii. [selecting] isolating a subset of said nucleic acid fragments;

wherein either (i) or (ii) or both (i) and (ii) are done in a sequence-dependent manner.